

GnRH Single-Injection Immunocontraception of Black-Tailed Deer

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ABSTRACT: High deer densities increase vehicle collisions, damage agricultural crops, and amplify the spread of zoonotic and animal diseases, intensifying human-deer conflict. In addition, deer impact on forest vegetation can influence the distribution and abundance of other wildlife species. Greater demand for non-lethal means of animal damage control has led to an interest in contraception as a wildlife management tool. The development of a single-injection Gonadotropin-Releasing Hormone (GnRH) contraceptive vaccine by NWRC reduces logistical limitations and cost of using immunocontraception as compared to a vaccine that requires two injections. This study assessed the efficacy of two different GnRH-KLH (keyhole limpet hemocyanin) vaccine designs in a single-injection study, to determine if *Mycobacterium avium* bacterium in the adjuvant is necessary for the success of a single-injection contraceptive vaccine. Forty-two captive female black-tailed deer were divided into 3 groups. Control deer were injected with saline solution, one treatment group received GonaCon™ (a GnRH-KLH vaccine paired with AdjuVac™ adjuvant that contains a small quantity of killed *M. avium* bacterium), and the second treatment group received GnRH-KLH vaccine with DEAE-Dextran/oil as the adjuvant. Contraceptive success was evaluated by monitoring progesterone, pregnancy specific protein, antibodies to GnRH-KLH conjugate and to Johne's bacterium (*M. avium*), and actual pregnancy rates. Pregnancy rates were significantly different based on treatment ($X^2 = 9.389$; $df = 2$; $P = 0.009$). Pregnancy rates in deer treated with GonaCon™ were significantly reduced as compared to saline controls ($P = 0.006$), but there was no significant difference between GnRH-DD compared to saline ($P = 0.297$). Significant difference was found between GonaCon™ and GnRH-DD ($P = 0.055$). Results suggest that *M. avium* in the AdjuVac™ adjuvant is essential for the success of the single-injection GnRH vaccine GonaCon™. The development of a single-injection vaccine will increase the practicality and lower the cost of using immunocontraception as a tool to control deer populations.

KEY WORDS: AdjuVac™, black-tailed deer, contraception, GnRH, GonaCon™, immunocontraception, *Odocoileus hemionus columbianus*, wildlife population control

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INTRODUCTION

People value deer (*Odocoileus* spp.) for a variety of reasons; they provide opportunities for hunting, wildlife viewing, photography, and some people simply value that they exist. Unfortunately, concerns pertaining to deer-human conflict are prevalent as well (Stout *et al.* 1993, Conover 1997), particularly where population densities are high. Columbian black-tailed deer (*Odocoileus hemionus columbianus*) is one of several deer species whose overabundance results in the potential to cause both biological problems and human-wildlife conflicts (McShea *et al.* 1997).

Deer damage a wide variety of agricultural crops, including grain, forage, vegetable, and ornamental crops (Craven and Hygnstrom 1994). Deer can be a detriment to reforestation efforts in the Pacific Northwest (Rochelle 1992) due to growth suppression, regeneration delay, and seedling mortality caused by repeated browsing (Crouch 1976, Tilghman 1989). Over-browsing by deer has a negative impact on the viability of forest plants. American ginseng (*Panax quinquefolius*) (McGraw and Furedi 2005) and other valuable understory herbs are at risk of extinction (Miller *et al.* 1992, Rooney and Gross 2003, McGraw and Furedi 2005) if deer densities remain high. In addition to threatening plant species, studies demonstrated that deer density impacts songbird abundance (deCalesta 1994, McShea and Rappole 2000).

Over-browsing by deer results in reduced habitat, including food resources and nest sites for forest birds. Species that depend on understory vegetation are more likely to experience a population decline, due to a decrease in plant density and diversity created by browsing of overabundant deer (deCalesta 1994, McShea and Rappole 2000, Allombert *et al.* 2005).

High deer densities are generally correlated with increased disease transmission (Klein 1981, Davidson and Doster 1997). There is rising concern that high deer densities threaten free-roaming wildlife as well as farmed game and livestock through the transmission of bovine tuberculosis (B.T.) (Schmitt *et al.* 1997, Palmer *et al.* 2000, Mackintosh *et al.* 2004) and chronic wasting disease (CWD) (Miller *et al.* 2004). Zoonotic diseases such as Lyme disease have also been increasing over the years (CDC 2004). Additional human health risks include injuries or death resulting from deer-vehicle collisions. It is estimated that over 1 million deer-vehicle collisions occur annually in the United States paired with an estimated \$1.1 billion spent in repairs (Conover *et al.* 1995). Survey response revealed that Lyme disease and deer-vehicle collisions were the most prevalent concerns related to deer in suburban environments (Connelly *et al.* 1987).

Herd reduction is a management option that frequently surfaces as a tool to decrease problems associated

with deer. However, decreased public support for hunting (Brown *et al.* 2000), an overall decrease in hunters (Enck *et al.* 2000, Riley *et al.* 2003), hunter unwillingness to hunt antlerless deer (McShea *et al.* 1997), decreased hunter access to lands (Brown *et al.* 2000, Wright *et al.* 2001), and safety concerns regarding firearms in urban areas may limit hunting as a primary means to reduce deer numbers in overpopulated areas. A case study review of 12 communities addressing deer management issues revealed that 10 of the communities opposed lethal methods, while others voiced concern that non-lethal methods would be too expensive and questioned their efficacy. Common conclusions among the communities revealed that a combination of lethal and non-lethal methods may be desirable (Raik *et al.* 2005).

Multiple non-lethal approaches to minimize deer damage have been attempted (Nolte 1999). Fencing that excludes ungulates from problem areas is an effective tool (Craven and Hygnstrom 1994), but it is expensive to install and maintain. Individual barriers work well (Nolte 1999), and some repellents may reduce deer browsing for a few months (Wagner and Nolte 2001). Although these non-lethal tools may be effective, they have limited application when managing overabundant deer. When trying to find solutions to manage deer populations, relocation emerges as a non-lethal method with public appeal. However, relocating deer is expensive, stressful for the deer, and lethal to most of the animals (O'Bryan and McCullough 1985, Cromwell *et al.* 1999, Beringer *et al.* 2002). Additional non-lethal approaches need to be identified.

Deer management strategies are necessary to respond to complaints about deer-vehicle collisions, transmission of diseases, damage to ornamental plants and agricultural crops, as well as to address ecological impacts of over-browsing by deer on native plant and bird habitat. Greater demand for non-lethal management methods has led to an interest in wildlife fertility control.

Using population modeling, Dolbeer (1998) compared the relative efficacy of sterilization to lethal removal in reducing wildlife populations. Since deer do not usually reproduce their first year, have a small number of offspring, and have a life span of 10-12 years, sterilization would be less effective in reducing their population than lethal removal (Dolbeer 1998). Sterilization is best suited for species with a short life span and a high reproductive rate (Dolbeer 1998, Miller and Fagerstone 2000). Seagle and Close (1996) used population modeling based on white-tailed deer (*Odocoileus virginianus*) population estimates to simulate the results of contraception as a management tool. They found that sterilization of <50% of the does would maintain population size over a 30-year period. Sterilization of >50% of the does in a closed population would need 5-10 years to show a significant population decline (Seagle and Close 1996). There is increasing evidence that white-tailed does have strong site fidelity. High-altitude populations may move between winter and summer ranges; however, deer residing in urbanized areas have been documented to inhabit home ranges of 40-170 ha, which is smaller than home ranges of deer residing in forested or agriculture landscapes (Kilpatrick and Spohr, 2000, Rutberg *et al.* 2004). Strong

site fidelity and smaller home ranges offer greater opportunity for contraception as a management tool for urbanized deer populations, without immigration completely negating the benefits. Sterilization may be most effective to maintain deer herd size after initial herd reduction via another means (Neilson *et al.* 1997, Miller and Fagerstone 2000), when a long-term population decline is desirable, or to prevent overabundance in a population.

Unlike in white-tailed deer, there is not a widespread demand for reducing populations of black-tailed deer; however, there is interest in reducing their populations in problem areas. Island communities with a somewhat closed population, residential areas, public parks, game parks, and zoos are currently the most applicable cases for the use of immunocontraception vaccines to help maintain lower black-tailed deer densities.

Traditional immunocontraceptive research in mammals has concentrated on the use of a vaccine called porcine zona pellucida (PZP) (Miller *et al.* 1999). Animals that are immunocontracepted with PZP continue to cycle and may copulate, but do not become pregnant due to the PZP antibody coating the egg. In polyestrous animals, such as deer, that continue to cycle throughout the breeding season, PZP contraception may introduce physiological stress due to an extended breeding season (Miller and Killian 2000). This prolonged estrous cycling results in increased activity during early winter, a time when conservation of calories is essential. Increased activity may also contribute to increased collisions with automobiles. Due to the concerns associated with prolonged estrous cycling, NWRC developed a second contraceptive that reduces reproductive behavior (Miller *et al.* 2004).

Gonadotropin-releasing hormone (GnRH) is a small peptide hormone that controls reproductive processes in both males and females. Identical in all mammals, the GnRH peptide is not immunogenic. However, coupling GnRH to a large foreign carrier protein, such as keyhole limpet hemocyanin (KLH), overcomes immune system tolerance to GnRH and results in a highly immunogenic GnRH conjugate that can then be combined with an adjuvant to create an immunocontraceptive vaccine. Immunization against the GnRH hormone prevents the circulating GnRH from stimulating the release of pituitary luteinizing hormone (LH) and follicle-stimulating hormone (FSH), which are responsible for sperm production in males and follicular development and ovulation in females, resulting in temporary non-surgical contraception in both male and female mammals (Miller *et al.* 2000). GnRH contraceptive vaccines have been evaluated as immunocastration agents in many species, including pets, cattle, sheep, swine, and deer (Adams and Adams 1992, Meloan *et al.* 1994, Ladd *et al.* 1994, Oonk *et al.* 1998). All of these vaccines have required 2 or more injections, and the duration of efficacy was 6 months or shorter, which is not practical for wildlife management uses.

A key ingredient in vaccine development is the adjuvant, which is responsible for alerting the immune system to be on the lookout for infection and which also continually stimulates the immune system to produce

antibodies. A water-soluble polycationic diethylamino-ethyl ether polymer of dextran (DD) has been shown to be a potent adjuvant for vaccines (Houston *et al.* 1976, Joo and Emod 1988). In a study of GnRH-ovalbumin conjugate vaccine in white-tailed deer, DD was used as the adjuvant for multiple injections to elicit an immune response against GnRH (Becker *et al.* 1999); however, multiple-injection vaccines are not practical for most wildlife applications. The new adjuvant developed (AdjuVac™) uses a small quantity of *Mycobacterium avium*, a generally nonpathogenic bacterium commonly found in domestic and wildlife species (Miller *et al.* 2004). *M. avium* is used in a USDA-approved Johne's disease vaccine. The KLH-GnRH conjugate combined with the new adjuvant AdjuVac™ formed the basis of the new multi-year single-injection GnRH vaccine GonaCon™ (Miller *et al.* 2004). No studies of a single-injection GnRH conjugate vaccine had been performed using an adjuvant that did not have a bacterial component. GonaCon™ has been shown to reduce breeding behavior and fawning in white-tailed does for up to 4 years (Miller and Killian 2000).

One feasible non-lethal approach to reduce deer numbers is to sterilize segments of target populations. Surgical sterilization, although permanent and effective, is very expensive and impractical, and therefore it has limited applicable use. The cost of administering contraceptive vaccines by darting free-ranging deer may be prohibitive as a management tool (Nielson *et al.* 1997), in particular due to the need of administering a booster injection to the same animal. The development of a single-injection vaccine would increase the practicality and lower the cost of immunocontraception as a management tool.

A need to reduce reproductive rates of black-tailed deer held at the National Wildlife Research Center (NWRC) Olympia Field Station (OFS) prompted a request to sterilize part of the herd. An experimental design was applied to this management objective to enable collection of data on black-tailed deer responses to immunocontraceptive vaccines and to assess the contraceptive efficacy of single-shot GnRH conjugate vaccine with diethylaminoethyl (DEAE)-Dextran (GnRH-DD) emulsified in oil as the adjuvant as compared to the GonaCon™ vaccine.

METHODS

Vaccine

The GnRH-KLH vaccine construct was developed by the NWRC. The GnRH peptide hormone was made immunogenic by coupling the peptide to keyhole limpet hemocyanin (KLH) (Pierce Endogen, Rockford, IL). The GnRH used in this study was synthesized at Macromolecular Resources, Colorado State University (Fort Collins, CO).

The aqueous-based GnRH-KLH conjugate was combined in a 1:1 ratio by volume with a novel adjuvant (AdjuVac™), which is an oil-based modified USDA-licensed Johne's disease vaccine containing a small quantity of killed *M. avium* bacterium (Mycopar, Fort Dodge Animal Health, Fort Dodge, IA). The GnRH-

KLH/AdjuVac™ vaccine, also known as GonaCon™, has an APHIS USDA patent-pending status.

The second treatment vaccine was paired with the adjuvant diethylaminoethyl-Dextran (DD) (Sigma-Aldrich, St. Louis, MO), a polycationic derivative of Dextran. The aqueous-based GnRH-KLH conjugate bound to DEAE-Dextran was combined in a 1:1 ratio by volume with AdjuVac™ diluent.

Captive Black-Tailed Deer Study

The study was conducted at the OFS of the NWRC in Washington State. Forty-two adult female black-tailed deer reared at the OFS, individually identified by color- and number-coded ear tags, were selected for this study. The deer were stratified by age and then randomly assigned to one of 3 treatment groups, such that all ages were equally represented across groups. Age ranged from 1 to 6 years, with an average age of 3 years in each treatment group. The treatments included GonaCon™ vaccine (1000µg GnRH-KLH in AdjuVac™ adjuvant) ($n = 14$), GnRH-DD vaccine (1000µg GnRH-KLH in DD/oil adjuvant) ($n = 14$), and a saline control group ($n = 14$), and were administered on September 22 and 23, 2004. Deer handling facilities at the OFS consist of a series of holding boxes, leading to a Deerhandler™ squeeze chute (Delclayna Whitetail and Bison Co. Ltd., Swanville, MN). Deer were restrained and a 10-ml blood sample was collected. Serum was then separated for analysis of anti-GnRH and anti-Johne's antibody titers (*M. avium*), progesterone, and pregnancy-specific protein B (PSPB) levels. Does were then injected intramuscularly with their assigned treatments. All does were returned to their normal pastures and isolated from bucks for approximately 10 weeks to enable the vaccine to develop sufficient antibody titers. Treated does were penned with known fertile bucks on November 15, 2004 through March 20, 2005 to allow breeding.

Blood collections were repeated February 17, 2005 for assessment of anti-GnRH and anti-Johne's antibody titers, progesterone, and PSPB levels post vaccination. Due to mortality, one subject from each treatment group was eliminated from the trial prior to blood collection in February. All remaining deer were monitored for pregnancy, and fawn production was recorded for the 2005 season. Fawns were individually identified with ear tags and associated with individual does through physical and behavioral observations.

Anti-GnRH and anti-Johne's antibody titers were analyzed by treatment using an ANOVA with a post-hoc Tukey t-test (SAS Inc. 9.1., Cary, NC). Anti-GnRH antibody titers were compared to progesterone levels using Pearson's correlations. Actual pregnancy rates by treatment were compared using Pearson's Chi-Square test (SPSS Inc. 14.0 Chicago, IL). Fisher's Exact Test was used to further compare the efficacy of the treatments and to compare PSPB analysis to actual pregnancy rates.

A booster injection was administered in August 2005 to all of the fawn-producing does in the GonaCon™ treatment group and all of the subjects in the GnRH-DD group. For the next 4 years, all deer will continue to be monitored for pregnancy to assess multiple-year efficacy

Table 1. Post vaccine serum analysis*.

Treatment	GnRH Antibody Titers		Johne's Antibody Titers <i>M. avium</i>		Progesterone Levels		PSPB	Actual Pregnancy & Fawns
	9/2/04	2/17/05	9/2/04	2/17/05	9/2/04	2/17/05	2/17/05	Spring 2005
Saline	0 ± 0	0 ± 0	0.27 ± 0.11	0.25 ± 0.10	1.34 ± 0.30	6.58 ± 0.52	13 pos. 0 neg.	12 pregnant 18 fawns
GnRH – DD	0 ± 0	7.54 ± 4.87	0.13 ± 0.08	0.11 ± 0.06	2.32 ± 0.56	7.16 ± 0.91	10 pos. 3 neg.	10 pregnant 16 fawns
GnRH - Adjuvac™	0 ± 0	32.38 ± 13.33	0.20 ± 0.13	0.44 ± 0.14	1.24 ± 0.41	3.08 ± 1.02	5 pos. 8 neg.	5 pregnant 7 fawns

*Mean with Standard Error

of the immunocontraception vaccines.

Serum Analyses

Serum was assayed for GnRH antibodies by enzyme-linked immunoabsorbant assay (ELISA). Deer serum was tested at serial dilutions to endpoint titers. Two negative controls were run on each plate; one negative control was buffer without deer serum and the other was pre-vaccination deer serum. High-titer deer serum was used as the positive control. Serum samples were also analyzed for total progesterone by radioimmunoassay (RIA) according to the manufacturer's instructions (Coat-A-Count®, Diagnostic Products Corporation, Los Angeles, CA). Pregnancy was diagnosed through serum PSPB testing (Biotracking, Moscow, ID). PSPB is produced by the placenta, and therefore pregnant animals will have the protein in their blood. PSPB pregnancy detection has an overall accuracy rate of 97%.

RESULTS

None of the deer had detectable anti-GnRH antibody titers prior to treatment, and control deer had no detectable anti-GnRH titers post-treatment (see Table 1). Post-treatment anti-GnRH antibody titers differed significantly between treatment groups ($df = 2$; $P = 0.022$). Anti-GnRH antibody titers were higher in deer treated with GonaCon™, compared to both control deer and deer treated with GnRH-DD ($P < 0.05$). No significant difference was found in pre-treatment serum progesterone levels between groups ($df = 2$; $P = 0.164$). Post-treatment progesterone was significantly different based on treatment ($df = 2$; $P = 0.003$); further analysis showed progesterone levels were lower in deer treated with GonaCon™ as compared to both control deer and deer treated with GnRH-DD ($P < 0.05$). High post-treatment anti-GnRH antibody titers correlated with low post-treatment progesterone ($r = -0.537$, $P = 0.004$). Anti-Johne's antibodies were higher in deer vaccinated with GonaCon™ compared to deer vaccinated with GnRH-DD ($P < 0.05$). Mean anti-Johne's antibodies were approximately the same pre- and post-treatment for both the control and GnRH-DD treated deer (see Table 1). Mean anti-Johne's antibodies increased in the GonaCon™ group post vaccination.

Of 13 control deer, all were pregnant as diagnosed by PSPB tests. Of 13 deer treated with GonaCon™, 5 were pregnant and 8 were non-pregnant as diagnosed by PSPB tests. Of 13 deer treated with GnRH-DD, 10 were pregnant and 3 were non-pregnant as diagnosed by PSPB

tests. Fawning rates correlated highly with PSPB tests ($P < 0.001$). Actual pregnancy rates in deer were significantly different based on treatment ($X^2 = 9.389$; $df = 2$; $P = 0.009$). Pregnancy rates in deer treated with GonaCon™ were significantly reduced as compared to control ($P = 0.006$); there was no significant difference in pregnancy rates between GnRH-DD compared to control ($P = 0.297$). A significant difference in pregnancy rates was found between GonaCon™ and GnRH-DD ($P = 0.055$). Control deer produced 18 fawns or 1.4 fawns per doe, GnRH-DD deer produced 16 fawns or 1.2 fawns per doe, and GonaCon™ deer produced 7 fawns or 0.54 fawns per doe.

DISCUSSION

This study demonstrated that GonaCon™ was effective in reducing pregnancy rates of captive black-tailed deer. Results suggest that the killed *M. avium* in the AdjuVac™ adjuvant is essential for the success of the single-injection GnRH vaccine GonaCon™. The efficacy of *M. avium* may be attributed to the natural exposure of deer to *M. avium* bacteria in their environment: 34 of the deer in this study had antibodies to *M. avium* prior to vaccination. The continued exposure to *M. avium* in the environment may help initiate the continued immune response and high antibody titer levels necessary for multiple year contraception without the need of a booster injection. Deer are polyestrous, which may contribute to vaccine efficacy by initiating continued immune response to GnRH. Research has shown higher efficacy of GonaCon™ in white-tailed deer compared to the results of this study (Miller *et al.* 2000, Miller and Killian 2000). Perhaps the captive deer used in this trial had compromised immune systems, or possibly the 10-week period we allowed for immune response was not sufficient for the development of antibody titer levels necessary for contraception. The development of a single-injection vaccine will increase the practicality and lower the cost of using immunocontraception as a tool to control overabundant deer populations. Long-term field trials are necessary to better understand the biological effects of fertility control on populations of free-roaming deer.

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